

Transfer of entomopathogenic fungi among formosan subterranean termites and subsequent mortality

M. S. Wright, W. L. A. Osbrink and A. R. Lax

Formosan Subterranean Termite Research Unit, Southern Regional Research Center, ARS, USDA, New Orleans, USA

Ms. submitted April 3, 2000; accepted March 6, 2001

Abstract: Strains of the entomopathogens *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin were screened for effectiveness against the Formosan subterranean termite, *Coptotermes formosanus* (Shiraki). Biological control methods are being considered to augment current integrated pest management schemes that involve chemical baits and nonrepellent termiticides. In this study Formosan subterranean termites selected from four colonies in the field were exposed in the laboratory to three strains of *Beauveria bassiana* and two strains of *Metarhizium anisopliae*. The exposed termites were then allowed to mingle with an equal number of unexposed termites from the same nest and mortality of the combined group was determined. One week after exposure, *B. bassiana* strains, ATCC 26037 and ATCC 90519, had caused significantly higher mortality than the two *M. anisopliae* strains, ESC 1 and II B and the remaining strain of *B. bassiana*, ATCC 90518. The LT_{50} measurement for strains 26037 and 90519 was comparable at 1.8 and 2.0 days, respectively. Strains ESC 1 and 90518 caused 50% mortality at 6.5 and 11.0 days, respectively. Termites exposed to strain II B and the control group of termites did not reach 50% mortality during the 21 day experimental period. Exposure of termites to all fungal strains resulted in mortality in excess of that seen in the control groups of unexposed termites.

1 Introduction

Subterranean termites are estimated to cause \$1 billion in damage in the United States annually including prevention and repair costs. A predominant species, the Formosan subterranean termite (FST), *Coptotermes formosanus* (Shiraki), has become an economically significant pest in the United States in the past 50 years. Organochlorine compounds were previously used to control FST but their sale was banned in 1988. Replacement chemicals are not as effective (SU and SCHEFFRAHN, 1998). In addition, by disturbing soil around a structure, when landscaping or compensating for soil subsidence, the chemical barriers can be compromised and allow FST access to the structure (SU and SCHEFFRAHN, 1990). SU and SCHEFFRAHN (1998) review some alternative control methods including non-repellant termiticides and bait technology. In order for these techniques to work they must not repel termites, must be easily transferable in or on termite bodies and have delayed toxicity which allows transfer from foraging workers to members of the termite colony that do not forage (SU and SCHEFFRAHN, 1996, 1998). One alternative to chemical control, and the focus of this study, is the development and use of biological control agents. This work addresses fungal entomopathogens, but bacteria, viruses and protozoa also have potential as pathogenic agents. Fungi exhibit qualities which can make them ideal for this application, including a slow-acting nature similar to that of

successful chemicals, the ability to self-replicate, and the ability of fungal spores to be spread by termite social behavior (GRACE and ZOBERI, 1992). Pathogenicity of strains of both *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin have been demonstrated in laboratory colonies of *C. formosanus* (DELADE et al., 1995; WELLS et al., 1995). JONES et al., 1996 discovered that small numbers of *B. bassiana* and *M. anisopliae* spores can be spread throughout a *C. formosanus* colony without being detected by the termites. Conditions in a termite nest, moderate temperature and high humidity, are conducive to the growth of fungal species and are important factors in fungal survivability and propagation (KRAMM et al., 1982; IGNOFFO, 1992). Enzymes and compounds produced by the fungi also can affect their ability to pathogenize insects including termites. Destruxins, cyclic chemical compounds, have been discovered in *M. anisopliae* (KODAIRA, 1961) and are known to have varied effects on insects including neurotoxicity, immunodepressant activity and activation of insect muscle calcium channels (WAHLMAN and DAVIDSON, 1993). Both fungi in this study are known to produce cuticle-degrading enzymes that permit infection of the host insect without the need for consumption by the target organism. Three strains of *B. bassiana* and two strains of *M. anisopliae* were examined in this study to determine their transfer from exposed individuals to non-exposed subjects and the resulting rate of mortality.

2 Materials and methods

2.1 Propagation of fungal strains

Three strains of *B. bassiana* were obtained from the American Type Culture Collection (Rockville, Maryland) to provide cultures from three different original sources. Strain ATCC 26037 was isolated from the Colorado potato beetle in Czechoslovakia; strain ATCC 90518 was isolated from the soil in Oregon; and strain ATCC 90519 was isolated from the Japanese beetle. *Metarhizium anisopliae* strain ESC 1 was isolated by plating Bio-Blast termiticide powder (Eco-Science, East Brunswick, New Jersey) onto potato dextrose agar (PDA) (DIFCO, Detroit, Michigan). Bio-Blast was generously provided by Dr GREGG HENDERSON of Louisiana State University Agricultural Center (Baton Rouge, Louisiana). *Metarhizium anisopliae* strain II B was isolated in this laboratory by exposing strain ESC 1 to the termite nest volatile naphthalene and selecting colonies which exhibited apparent natural tolerance of the compound (unpublished data). All fungi were maintained on PDA in a 25°C incubator for 7 days then stored at 4°C.

2.2 Collection of termites

Formosan subterranean termites, *Coptotermes formosanus* Shiraki, were obtained from four colonies at the Southern Regional Research Center in New Orleans, Louisiana. Four colonies of termites were chosen to prevent colony vitality biasing of data. Each colony represented one replicate in the experiment. Bucket traps were established to allow access to termites (SU and SCHEFFRAHN, 1986). Twenty *C. formosanus* workers of at least 3rd instar (as determined by size) were used in each of the replicates. Termites were collected within 7 days of the beginning of the experiment. Soldiers were not included in the treatments because of the low proportion collected from the traps (table 1). Low soldier proportion is attributed to collection dates coinciding with swarming season. Workers were weighed in 4 groups of 10 termites for each colony (table 1).

2.3 Exposure of termites to fungi

Ten Formosan subterranean termites from each of four colonies were allowed to walk on fungal cultures for 10 min. These workers were then transferred to 100 × 15 mm Petri dishes (Falcon, Franklin Lakes, New Jersey) which contained Whatman #4 filter paper (Maidstone, England), dampened with sterile water (Solution 2000 Water Purification System, Solution Consultant Inc., Jasper, Georgia), and 10 unexposed worker termites from the same colony as those exposed to the fungus. Plates containing the combinations of exposed and unexposed termites were then placed in an unlit incubator at 25°C and 99% humidity. Control plates were incubated as described above and contained 20 termites, none of which had been exposed to fungal cultures. All work prior to incubation was conducted under a laminar flow hood (NuAire,

Plymouth, Minnesota). Experimental plates were monitored for 21 days. At each observation dead termites were counted and removed to PDA to allow growth of associated microbes on the cadaver. The Logistic Procedure (SAS Institute, Inc., 1989) was performed on termite mortality data to determine the median lethal exposure times (LT₅₀) for each fungal treatment.

3 Results

3.1 Overall percent mortality

Mortality data are presented for the end of each week during the experimental period. At 7 days *B. bassiana* strains 26037 and 90519 caused 100% mortality in every colony (fig. 1), indicating that all termites, including those not directly exposed to the fungi were killed. Exposure to *M. anisopliae* strain ESC 1 resulted in 69% mortality (fig. 1). As with the two *Beauveria* strains, mortality exceeding 50% indicates transfer of the fungus from exposed termites to their unexposed nestmates. *B. bassiana* strain 90518 and *M. anisopliae* strain II B caused 43% and 10% mortality at 7 days, respectively (fig. 1). The control termites, which had not been exposed to fungi, had an average mortality rate of 3% at 7 days (fig. 1). However, it is interesting to note that only one control colony, #24, showed any mortality (10%) (data not shown). All control termites from colonies 6, 18 and 19 were alive at 7 days post-exposure. At 15 days post-exposure, *M. anisopliae* strain ESC 1 had achieved 100% mortality in all termite colonies (fig. 1), *B. bassiana* strain 90518 and *M. anisopliae* strain II B caused 84% and 19% mortality, respectively (fig. 1). Among control termites the average mortality was 8% (fig. 1) with the majority of the dead again coming from colony 24 of which 15% had died (Data not shown). Observations made 21 days after exposure to the fungi revealed that *B. bassiana* strain 90518 had reached 96% average mortality, while only 25% of the termites exposed to *M. anisopliae* strain II B were dead (fig. 1). Among the control termites the average mortality was 14% (fig. 1), with colony 24 exceeding the other control group mortality rates at 35% (data not shown). Colony 6 control termites had 0% mortality after 21 days.

3.2 Lethal time

The time after exposure necessary to result in 50% mortality of termites by each fungus was determined. *B. bassiana* strains 26037 and 90519 averaged comparable values for the LT₅₀, 1.6 days and 1.4 days,

Table 1. Characteristics of the experimental *Coptotermes formosanus* colonies

Colony	Worker weight (Mean + SE (mg))	% Soldiers
6	3.12 ± 0.1	0.26
18	3.27 ± 0.4	0.04
19	2.48 ± 0.1	3.31
24	2.91 ± 0.1	0.80

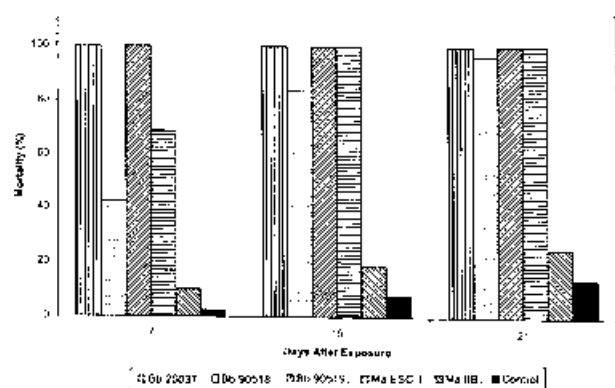


Fig. 1. Mean percent mortality at 7, 15 and 21 days after initial exposure of half of the experimental replicate to the specified fungus

respectively (fig. 2). *M. anisopliae* strain ESC 1 also gave an LT_{50} of under 1 week, 6.0 days (fig. 2). *B. bassiana* strain 90518 averaged an LT_{50} of 9.4 days. Fifty percent of the termites from colony 24 were dead at 15 days following exposure to *M. anisopliae* strain II B (data not shown). Fifty percent mortality was not achieved with any other colonies vs. *M. anisopliae* strain II B, nor with any of the control termites. After 21 days of observation greater than 25% mortality had not been reached among termites exposed to *M. anisopliae* strain II B, nor with any of the control termites.

3.3 Observation of fungi on cadavers

At each observation dead termites were counted, removed from the filter paper-lined Petri dish and transferred to another Petri dish containing potato dextrose agar to allow propagation of fungi associated with the cadaver. The average recovery of *B. bassiana* was 85% with termites exposed to strain 26037, 32% with strain 90518, and 74% with strain 90519 (table 2). *Metarhizium anisopliae* was observed on an average of 49% of dead termites exposed to strain ESC 1 and 74% of those exposed to strain II B (table 2). Neither *B. bassiana* nor *M. anisopliae* were recovered from the total of 11 control termite cadavers.

4 Discussion

Beauveria bassiana is known to infect termite species, specifically *Reticulitermes flavipes* (Kollar) (BAO and YENDOL, 1971) and *Heterotermes tenuis* (Hagen)

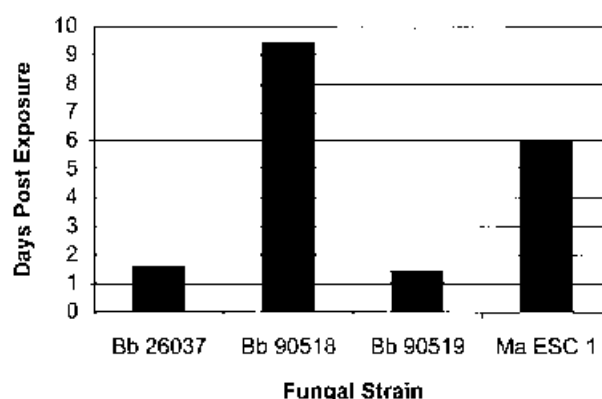


Fig. 2. Time, in days, necessary for the individual fungi to achieve an average of 50% mortality in all four experimental colonies. *B. bassiana* strains 26037, 90518, and 90519; *M. anisopliae* strain ESC 1

(ALMEIDA et al., 1997). Fungi are in general good candidates for inclusion in an integrated pest management scheme because, unlike bacterial and viral agents, fungi need not be ingested to cause infection (GUPTA et al., 1992). In addition, their activity can be enhanced by production of cuticle degrading enzymes (GUPTA et al., 1992). Several strains of *B. bassiana* have been studied and were discovered to have significantly different levels of cuticle degrading enzyme production (GUPTA et al., 1992). In a previous study SUZUKI (1995) found that one strain each of *M. anisopliae* and *B. bassiana* were effective against both *C. formosanus* and *Reticulitermes speratus* termites in the laboratory. DELATE et al. (1995) conducted a number of choice tests with one strain each of *M. anisopliae* and *B. bassiana* vs. *C. formosanus*. In all cases they found that *M. anisopliae* strain 2162 caused a higher mortality rate than *B. bassiana* strain 3041. In studying the effect of these two fungal species on *C. formosanus* WELLS et al. (1995) discovered that virulence varied widely between fungal strains within the same species. In addition, while the strain isolated from termites was most effective, isolates from insects closely related phylogenetically to *C. formosanus* were no more effective than isolates without a close phylogenetic relationship to the target insect (WELLS et al., 1995). This study compares the effectiveness of different strains of *B. bassiana* and *M. anisopliae* as entomopathogens.

It is theorized that termite social behaviour, trophallaxis and allogrooming, will aid the transfer of pathogenic fungi within the termite colony. Therefore,

Table 2. Percent of experimental fungi observed on termite cadavers

	Bb 26037	Bb 90518	Bb 90519	Ma ESC 1	Ma II B
Colony 6	90	18	70	35	100
Colony 18	75	65	80	50	50
Colony 19	85	25	80	75	100
Colony 24	90	20	65	35	46
Mean	85	32	74	49	74

spores picked up by foraging workers could be transferred from termite to termite, much as successful bait chemicals are spread. The observation of the fungal strains used in this study on the majority of termite cadavers, including those not originally exposed to the fungus, and the absence of those fungi on control termites supports this theory. A mortality rate of greater than 50% was seen here with exposure to *B. bassiana* strains 26037 and 90519, and *M. anisopliae* strain ESC 1. This evidence, in addition to the recovery of spores from the majority of termite cadavers, indicates transfer of fungi from exposed termites to unexposed individuals. It is also clear from this study that pathogenic variability exists among species. Among the *B. bassiana* strains, both 26037 and 90519 showed similar potential in overall mortality and lethal time observations. One replicate of *B. bassiana* strain 90518 did not achieve 100% mortality and all replicates took longer to achieve an LT₅₀ when compared to the other strains of the same species. Even more differentiation was seen when the two strains of *M. anisopliae* were compared. Strain ESC 1 had significantly higher overall mortality, 100% at 15 days, than did strain II B, 25% at 21 days. It is important to note that these strains are closely related. *M. anisopliae* II B was created by stressing ESC 1 with the volatile chemical naphthalene which is known to exist in Formosan subterranean termite nests (CHEN et al., 1998). It was theorized that this would naturally select spores that were capable of tolerating the chemical making them less subject to inhibition. This selection scheme will not be effective if a significant loss in pathogenicity also occurs. It will therefore be necessary to screen several cultures for an optimal combination of volatile tolerance and pathogenic potential.

Acknowledgements

The authors wish to thank Dr RICHARD M. JOHNSON and DEBBIE BOYKIN for statistical analysis; and BRIDGETTE C. HUNN and ERIN E. COURTNEY for technical assistance.

References

- ALMEIDA, J. E. M.; ALVES, S. B.; PEREIRA, R. M., 1997: Selection of *Beauveria* spp. Isolates for control of the termite *Heterotermes tenuis* (Hagen, 1858). *J. Appl. Entomol.* **121**, 539–543.
- BAO, L. L.; YENDOL, W. G., 1971: Infection of the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) with the fungus *Beauveria bassiana* (Balsamo) Vuill. *Entomophaga* **16**, 343–352.
- CHEN, J.; HENDERSON, G.; GRIMM, C. C.; LLOYD, S. W.; LAINE, R. A., 1998: Termites fumigate their nests with naphthalene. *Nature* **392**, 558–559.
- DELATE, K. M.; GRACE, J. K.; TOME, C. H. M., 1995: Potential use of pathogenic fungi in baits to control the Formosan subterranean termite. *J. Appl. Entomol.* **119**, 429–433.
- GRACE, J. K.; ZOBERI, M. H., 1992: Experimental evidence for transmission of *Beauveria bassiana* by *Reticulitermes flavipes* workers (Isoptera: Rhinotermitidae). *Sociobiology* **20**, 23–28.
- GUPTA, S. C.; LEATHERS, T. D.; EL-SAYED, N.; IGNOFFO, C. M., 1992: Insect cuticle-degrading enzymes from the entomogenous fungus *Beauveria bassiana*. *Exp. Mycol.* **16**, 132–137.
- IGNOFFO, C. M., 1992: Environmental factors affecting persistence of entomopathogens. *Florida Entomol.* **75**, 516–525.
- JONES, W. E.; GRACE, J. K.; TAMASHIRO, M., 1996: Virulence of seven isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Environ. Entomol.* **25**, 481–487.
- KODAIRA, Y., 1961: Toxic substances to insects produced by *Aspergillus ochraceus* and *Oospora destructor*. *Agric. Biol. Chem.* **25**, 261–262.
- KRAMM, K. R.; WEST, D. F.; ROCKENBACH, P. G., 1982: Termite pathogens: transfer of the entomopathogen *Metarhizium anisopliae* between *Reticulitermes* sp. Termites. *J. Invertebr. Pathol.* **39**, 1–5.
- SAS Institute Inc., 1989: SAS/STAT User's Guide, version 6, Fourth Edition, Volume 2. Cary, NC: SAS Institute, 1071–1126.
- SU, N.-Y.; SCHEFFRAHN, R. H., 1986: A method to access, trap, and monitor field populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the urban environment. *Sociobiology* **12**, 299–304.
- SU, N.-Y.; SCHEFFRAHN, R. H., 1990: Economically important termites in the United States and their control. *Sociobiology* **17**, 77–94.
- SU, N.-Y.; SCHEFFRAHN, R. H., 1996: Fate of subterranean termite colonies (Isoptera) after bait applications – an update and review. *Sociobiology* **27**, 253–275.
- SU, N.-Y.; SCHEFFRAHN, R. H., 1998: A review of subterranean termite control practices and prospects for integrated pest management programmes. *Integr. Pest Managem. Rev.* **3**, 1–13.
- SUZUKI, K., 1995: Biological control of termites by pathogenic fungi. Conference Forestry and Forest Prod. Res. 146–156.
- WAHLMAN, M.; DAVIDSON, B. S., 1993: New destruxins from the entomopathogenic fungus *Metarhizium anisopliae*. *J. Nat. Prod.* **56**, 643–647.
- WELLS, J. D.; FUXA, J. R.; HENDERSON, G., 1995: Virulence of four fungal pathogens to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *J. Entomol. Sci.* **30**, 208–215.

Authors' address: M. S. WRIGHT (corresponding author), W. L. A. OSBRINK, A. R. LAX, Formosan Subterranean Termite Research Unit, USDA-ARS-SRRC 1100 Robert E. Lee Blvd., New Orleans LA 70124, US. E-mail: mswright@nola.srrc.usda.gov